ECOLOGY AND CYTOLOGY OF SOME ALBERTA BLACK FLIES (DIPTERA: SIMULIIDAE)

Peter H. Adler[1]
Department of Entomology
University of Alberta
Edmonton, Alberta T6G 2E3
CANADA

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ABSTRACT

Sibling species of five black fly complexes in Alberta, Canada, were studied cytologically and ecologically during summer 1984. Species complexes (and cytological entities) were Simulium aureum *(A, B, D),* S. vittatum *(IIIL-1, IS-7),* S. tuberosum *(AB, FG, FGH, FGI),* S. venustum *(CC, CC3, CC4), and* S. verecundum *(ACD). Possible hybridization within each species complex was 0.3% or less, except in some populations of the* S. venustum *complex where extent of hybridization was not resolvable. CC4 was provisionally designated as a new cytotype of* S. venustum *to accommodate larval populations predominantly standard for IIIL-5. Two additional species,* Cnephia dacotensis *and* S. decorum, *were conspecific with respective populations in eastern North America. Habitat partitioning occurred within the* S. vittatum *and* S. tuberosum *complexes and between* S. verecundum *ACD and the* S. venustum *complex. The first example of three sibling species partitioning habitat was recorded for* S. tuberosum *FGH, FG, and AB. Results of this study conform to the pattern that the most closely related black fly species coexist by partitioning habitat along the river continuum.*

RÉSUMÉ

La cytologie et l'écologie des espèces soeurs de cinq complexes de mouches noires de l'Alberta, Canada, ont été étudiées pendant l'été 1984. Les complexes d'espèces (et les entités cytologiques) considérés ici sont Simulium aureum *(A, B, D),* S. vittatum *(IIIL–I, IS–7),* S. tuberosum *(AB, FG, FGH, FGI),* S. venustum *(CC, CC3, CC4), et* S. verecundum *(ACD). Les possibilités d'hybridation parmi les espèces d'un même complexe sont de 0.3% ou moins, sauf chez certaines populations du complexe de* S. venustum *pour lesquelles il ne faut pas possible de déterminer le niveau d'hybridation. Un nouveau cytotype de* S. venustum, *qui comprend des populations de larves correspondant de façon prédominante au standard de IIIL–5, est provisoirement désigné CC4. Deux espèces additionnelles,* Cnephia dacotensis *et* S. decorum, *sont conspécifiques avec leurs populations respectives de l'est de l'Amerique du Nord. Le morcellement de l'habitat existe parmi les espèces des complexes de* S. vittatum *et de* S. tuberosum, *et entre* S. verecundum *ACD et le complexe de* S. venustum. *Un premier cas de morcellement de l'habitat chez les espèces soeurs du complexe de* S. tuberosum *a été découvert entre les cytotypes FGH, FG, et AB. Les résultats de cette étude confirment que les espèces de mouches noires les plus proches parentes coexistent en morcelant l'habitat le long du continuum riverain.*

INTRODUCTION

Presence of sibling species (cytospecies) within the Simuliidae has necessitated a re-evaluation of base-line biological data for many of the most common black flies. Geographic regions must be resurveyed and biological and ecological profiles drawn for each sibling species. Only a few regional surveys of black-fly sibling species have been conducted in North America

[1]Department of Entomology, Clemson University, Clemson, SC 29634–0365, U.S.A.

(Adler and Kim 1986; Currie and Adler 1986), although a number of cytological studies have provided distributional information for sibling species. A few ecological studies of individual sibling species have been undertaken in eastern North America (Gordon and Cupp 1980; Lake and Burger 1983; Adler and Kim 1984).

The present study provides ecological, cytological, and distributional data for two species and the constituent siblings of five complexes in Alberta. The morphospecies of Alberta have recently been surveyed (Currie 1986), providing a basis for the current study. A few collection records from Alberta have been given previously for siblings in the *Metacnephia pallipes* (Fries), *Simulium canonicolum* (Dyar and Shannon), *S. vernum* Macquart, *S. vittatum* Zetterstedt, *S. tuberosum* (Lundström), and *S. venustum* Say and *S. verecundum* Stone and Jamnback complexes (respectively: Procunier 1982a; Golini and Rothfels 1984; Brockhouse 1985; Rothfels and Featherston 1981; Mason 1984; Rothfels *et al.* 1978). The most detailed studies of Alberta sibling species pertain to the *S. arcticum* Malloch complex (Shields and Procunier 1982; Procunier 1984). The present study examines cytological and ecological aspects of the following species and species complexes in Alberta: *Cnephia dacotensis* (Dyar and Shannon), *S. aureum* Fries, *S. vittatum*, *S. decorum* Walker, *S. tuberosum*, *S. venustum* and *S. verecundum*.

PRINCIPAL STUDY SITES

The two principal study sites were located on White Mud Creek, a third order stream in central Alberta (53°20' N, 113°30' W). White Mud Creek arises from several intermittent ponds southwest of Leduc, and drains predominantly agricultural (cereal and forage) cropland in a level to gently undulating glacial-till plain. Site 1 was located at the junction with Highway 19, just east of Leduc. Site 2 was immediately downstream from a beaver dam, approximately 1.4 km north (downstream) of site 1, and between the confluence with Black Mud Creek and the 23 Avenue Bridge. Black Mud Creek originates from Saunders Lake, east of Leduc. Sites 1 and 2 were less than 10% shaded. Riparian vegetation was predominantly low herbaceous growth, although areas upstream and downstream of both sites were bordered by aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), and numerous shrubs.

MATERIALS AND METHODS

Principal study sites were sampled qualitatively for black fly larvae on 2-3 June 1984 and (by D. C. Currie) on 8 and 26 May 1985. Weekly in 1984, from 10 June until flow ceased (31 July at site 1; 5 August at site 2), sites were sampled quantitatively, and selected physical and chemical parameters of the stream were measured. Five stratified random samples (strata within flowing water: rocks and grasses) within a 15-m stretch of stream per site were taken with a Surber sampler (area = 0.1 m^2). Sample size was proportionally reduced when less than 15 m of the site was available for black fly larvae. The sampler was placed firmly into the stream bed, and individual pieces of substrate were removed and examined for larvae. Larvae (middle through ultimate instars) were removed with forceps and placed into Carnoy's fixative (1 part glacial acetic acid: 3 parts 95% ethanol). Remaining substrate was agitated by hand for one minute and additional larvae were removed from the sampling net and placed into the fixative, which was renewed three to five times within two hours of sampling.

Table 1. Collections of larval simuliids from Alberta. Refer to Fig. 1 for relative locations.

Site	Ecoregion[a]	Location	Collector[b]	Date[c]
1–2	P	Edmonton, see text under Study Sites	PA	See text
3	P	trib., White Mud Creek, Hwy 39 (53° 15′N, 113° 37′W)	PA	24, 30 June
4	P	Donsdale Creek, 184 Street, Edmonton (53° 32′N, 113° 36′W)	PA	2 June-5 Aug.
5	P	Sturgeon River, Hwy 635, near Onoway (53° 44′N, 114° 16′W)	PA	2 June-5 Aug.
6	P	outlet, George Lake, near Dunstable (53° 56′N, 114° 07′W)	RA	18 June
7	P	Norris Creek, Hwy 16, 22 km north of Tofield (53° 34′N, 112° 42′W)	WB	11 May
8	P	Vermilion River, 1 km west of Vegreville (53° 29′N, 112° 02′W)	WB	12 May
9	F	Red Deer River, Hwy 11 bridge (52° 15′N, 113° 36′W)	GB	17 July
10	P	Red Deer River, Drumheller, Hwy 9 bridge (51° 28′N, 112° 42′W)	GB	18 July
11	M	Sibbald Flat, Sibbald Creek Trail (50° 56′N, 114° 46′W)	PA,DC	26 July
12	M	Lake Minnewanka (west end at dam), near Banff (51° 14′N, 115° 29′W)	PA	27 July
13	F	Chungo Creek, north of Blackstone River, Forestry Trunk Road (52° 42′N, 116° 19′W)	PA	29 July
14	F	Brown Creek, Forestry Trunk Road (52° 46′N, 116° 23′W)	PA	29 July
15	F	1st stream north of Brown Creek, Forestry Trunk Road (52° 50′N, 116° 27′W)	PA	29 July
16	F	Pembina River, Forestry Trunk Road (52° 56′N, 116° 34′W)	PA	29 July

(continued on next page)

Table 1 (continued)

Site	Ecoregion[a]	Location	Collector[b]	Date[c]
17	F	Lovett River, Forestry Trunk Road (53° 00′N, 116° 40′W)	PA	29 July
18	W	Pembina River, jct. Hwy 16 (53° 37′N, 115° 00′W)	PA	27 June
19	F	Sundance Creek, jct. Hwy 47 (53° 33′N, 116° 35′W)	PA,DC	15 June
20	F	3.2 km east of Obed summit (53° 31′N, 117° 17′W)	PA	15, 27 June
21	F	Hwy 16, 3.1 km west of Obed exit (53° 33′N, 117° 14′W)	PA,DC	11, 27 June
22	F	Sundance Creek, jct. Hwy 16, near Hornbeck (53° 34′N, 116° 38′W)	PA,DC	11, 27 June
23	F	Hwy 16, 10 km east of Marlboro (53° 33′N, 116° 45′W)	PA,DC	15 June
24	W	Sakwatamau River, Hwy 32, 1st crossing south (54° 12′N, 115° 47′W)	PA,DC	2 August
25	W	Sakwatamau River, Hwy 32, 2nd crossing south (54° 10′N, 115° 43′W)	PA,DC	2 August
26	W	Paddle River, Barrhead (54° 07′N, 114° 24′W)	PA	8 July
27	W	Hwy 33, 20 km north of Hwy 658 (54° 30′N, 115° 04′W)	PA	8 July
28	F	Hwy 33, 34 km north of Hwy 658 (54° 36′N, 115° 12′W)	PA	8 July
29	F	Freeman River, Hwy 32 (54° 34′N, 115° 24′W)	PA,DC	2 August
30	F	trib., Morse Creek, Hwy 33, 50 km north of Hwy 658 (54° 40′N, 115° 17′W)	PA	8 July
31–35	F	near Edith Lake, Swan Hills, off Hwy 33 (54° 48′N, 115° 23′W)	PA,DC	2 August
36	F	Morse River, Hwy 32, south of town of Swan Hills (54° 41′N, 115° 24′W)	PA,DC	2 August
37	F	Hwy 33, 37 km north of Hwy 658 (54° 37′N, 115° 14′W)	PA	8 July

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Table 1 (continued)

Site	Ecoregion[a]	Location	Collector[b]	Date[c]
38	W	Hwy 33, 30 km north of Hwy 658 (54° 35′N, 115° 10′W)	PA	8 July
39	W	Baptiste Creek (near mouth), near town of Athabasca (54° 44′N, 113° 32′W)	GB	26 July
40	W	Muskeg Creek, south of Athabasca (54° 35′N, 113° 25′W)	GB	3 August
41	W	Deep Creek, Hwy 813, north of Athabasca (54° 52′N, 113° 16′W)	GB	23 July
42	W	Parallel Creek (near mouth), south of Pelican Portage (55° 47′N, 112° 38′W)	GB	25 July
43	W	unnamed creek, south of Pelican Portage (55° 46′N, 112° 37′W)	GB	1 August
44	W	Pelican Creek (near mouth), north of Pelican Portage (55° 50′N, 112° 39′W)	GB	24 July
45	W	Poplar Creek, Hwy 16, near Tar Island (56° 55′N, 111° 28′W)	GB	5 July
46	W	West Interception Ditch, near Mildred Lake (57° 06′N, 111° 41′W)	JC	18 June
47	W	Poplar Creek, 1.5 km west of Hwy 63 (56° 55′N, 111° 29′W)	JC	18 June

[a] F, Foothill; M, Montane; P, Parkland; W, Mixedwood (Currie 1985).
[b] PA, P. H. Adler; RA, R. B. Aiken; WB, W. B. Barr; GB, G.Byrtus; JC, J. J. H. Ciborowski; DC, D. C. Currie.
[c] all collections 1984, except sites 7-8 (1985).

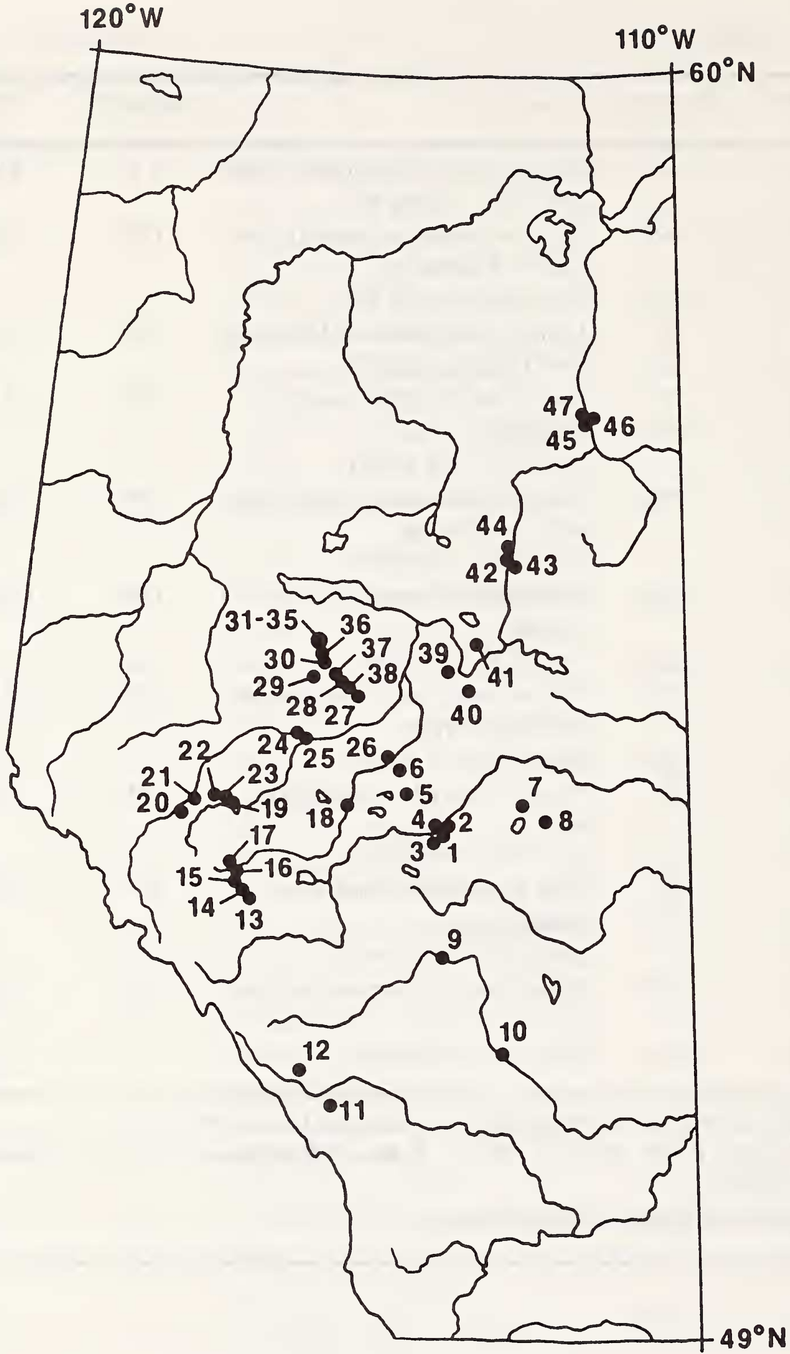

Figure 1. Map of Alberta, showing major rivers and 47 collection sites of larval black flies. Refer to Table 1 for specific locations and dates.

Table 2. Selected physical and chemical characteristics for 3 sample dates during July 1984 at site 4, Alberta. Black fly composition (n = 996): *S. verecundum* ACD (70.6%), *S. vittatum* IIIL-1 (19.1%), *S. aureum* A (4.7%), *S. tuberosum* AB (2.9%), *S. tuberosum* FG (1.9%), and *S. aureum* B (0.8%).

	Median	Minimum-Maximum
Temperature (°C)	16	4 – 19[a]
Conductivity (μS cm^{-1})	110	100 – 125
pH	8.30	8.26 – 8.33
Dissolved oxygen (mg l^{-1})	11.0	9.9 – 11.1
Minimum width (m)	0.6	0.3 – 0.8
Maximum width (m)	2.7	2.4 – 2.7
Depth (cm)	5.7	2.5 – 10.0
Seston (mg l^{-1})	12	2 – 16
Velocity(cm sec^{-1})	34	14 – 58

[a] measured with minimum-maximum thermometer

Because larvae were nearly always contagiously distributed, median densities with nonparametric confidence intervals (CI) are reported. An approximate 90% CI is given for sample sizes of four and an approximate 95% CI for sample sizes greater than four. These intervals, based on the sign test, are two-sided and symmetrical (Daniel 1978). Median, minimum, and maximum densities are reported for sample sizes less than four. Densities reflect number of larvae per m^2 of available habitat. These data must be interpreted in view of habitat reduction over time. Site 1 decreased in area from 105 m^2 of available habitat (10 June) to 7.2 m^2 (23 June). Site 2 declined from 142 m^2 (10 June) to 1.5 m^2 (7, 14 July).

Temperature was measured with a minimum-maximum thermometer, conductivity with a Yellow Springs Instrument Company conductivity meter, pH with a Fisher digital pH meter model 109, and oxygen with a Yellow Springs Instrument Company oxygen meter model 51B. Minimum and maximum stream widths within the 15-m sampling stretch were measured. Maximum widths often included large pools of standing water. Stream depth and velocity were measured in the center of each sampling unit. Velocity was determined using the formula $v^2 = 2hg$, where h = [(distance water rises up a meterstick when perpendicular to current)-(depth of water)] and g = gravitational acceleration (Newbury 1984). Seston load was measured in the field by filtering 500 ml of stream water through a 0.40 μ Millipore® filter mounted in a portable, hand-operated vacuum pump. Pre-weighed filters with filtrate were dried at 65°C for 24 h and weighed to the nearest mg.

Qualitative or quantitative samples of black fly larvae were collected at 45 additional sites throughout Alberta from June to mid-August 1984 and in May 1985 (Table 1, Fig. 1). Temperature (mid-day), conductivity, pH, dissolved oxygen, seston load, and width were measured at many of these sites.

The Feulgen method (Rothfels and Dunbar 1953) was used to prepare salivary gland chromosomes of larvae from all collections. For samples with individual morphospecies represented by over 30 larvae, a random subsample of 22 larvae was prepared. Preparations were examined under oil immersion with a Leitz SM-LUX compound microscope, identified to sibling, and scored for various cytological features. Representative slide mounts of chromosomes are housed in the laboratory of K. Rothfels, University of Toronto.

All visually detectable pathogens and parasites were recorded. Temporary smears of microsporidia from fresh and Carnoy's-preserved larvae were examined. Fresh smears were air-dried, fixed in absolute methanol, stained with Giemsa's stain, and made permanent in Euparal®. Representative mounts were deposited in the Department of Entomology, University of Alberta.

TAXA AND ECOLOGICAL CONSIDERATIONS

Cnephia dacotensis (Dyar and Shannon)

Larvae of this univoltine species occurred early in the season; final instars were found as early as the first week of May at site 2. Larvae present on 2 June at sites 1 and 2 represented the end of the major hatch. Small populations from mid- to late June probably represented late-hatching eggs (Tables 3, 4). Larvae conforming morphologically and cytologically to *C. dacotensis* were also collected at sites 6-8. At all sites, larvae occurred in organically enriched flows not exceeding 21°C.

Sex-chromosome constitution of 12 larvae from site 2 was X_3 Y_0 (n=3), X_0 X_3 (n=2), and X_3 X_3 (n=7) [terms of Procunier (1982b)]. Floating inversions and B chromosomes were lacking from these larvae.

Simulium aureum cytospecies A, B, and D

Cytospecies A and B were recorded, for the first time from Alberta, at site 4. One mature larva of cytospecies D was found at site 46. Cytological information from these collections was integrated with that from other North American collections (Leonhardt 1985). Larvae of all siblings were collected exclusively from trailing grasses. Larvae of A and B were collected in mid-July, but were absent throughout June and from late July through early August. Median larval densities (with ca. 90% CI) on 14 July were 60 m^{-2} (0-350 m^{-2}) for A and 10 m^{-2} (0-50 m^{-2}) for B. On 22 July, no larvae of A and 2 of B were collected in 5 samples (each 0.1 m^2). Chemical and physical data recorded for the duration of the *S. aureum* A and B generations are given in Table 2.

S. decorum Walker

Larvae from sites 1-4 conformed cytologically to eastern North American collections in having a heavy band in the base of IL and identical sex chromosomes. Population trends at sites 1 and 2 indicated a generation each for June and July (Tables 3, 4). The first generation probably occurred in May, as suggested by presence of larvae in a qualitative sample from site 2 on 8 May 1985. One 0.1 m^2 sample of 99 larvae was collected at site 3 on 24 June, at which time only 1 m^2 of flowing water was present. Values of all physical and chemical parameters measured during June at site 3 fell within the range experienced by *S. decorum* at sites 1 and 2 (Tables 3, 4), with the exception of conductivity (27-30 $\mu S\ cm^{-1}$), width (0.2-0.5 m), and seston (2-8 mg l^{-1}).

Patent microsporidian infections [*Pleistophora multispora* (Strickland)] occurred in 1.0% of the population at site 1 and in 13.1% at site 3. Ants of the *Formica fusca* L. group preyed on teneral adults emerging at site 3.

***S. tuberosum* cytospecies AB**

This multivoltine sibling was abundant throughout the province. Larvae occurred in large numbers at site 2 from 3 June to 7 July (Table 4). In 1985, late instars were common on 8 May at site 2, but virtually absent on 26 May. Only one AB larva was found at site 1 (30 June). Site 4 supported a population during early June, one in mid-July (median, ca. 90% CI = 75 m^{-2}, 50-90 m^{-2}; as measured 14 July), and another that appeared in early August. Additional populations of AB occurred at sites 8-10, 14, 16, 18, 24-29, and 40-44. Flows in which AB was collected varied from 12-125 μS cm^{-1} in conductivity, from 7.78 to 8.50 in pH, and from 2-36 mg l^{-1} in seston. AB was the exclusive member of the *S. tuberosum* complex in flows exceeding 22°C. Consequently, AB generally predominated in rivers greater than 10 m in width. More than 75% of the watercourses from which it was collected were wider than 10 m. AB occurred, as well, in streams with an average width down to approximately 2 m; these smaller streams were often organically enriched.

Less than 0.2% of larvae at site 2 were infected with a microsporidium [*Thelohania varians* (Léger)]. One male larva each from sites 16 and 41 harbored patent infections of *Coelomycidium simulii* Debaisieux.

B chromosomes occurred in approximately 3% of male and female larvae. B's in the AB sibling have been reported from other northern collections (Mason 1984). The pale larval head capsule and body generally permitted morphological identification of cytospecies AB. Final-instar larvae (n = 21) from sites 2, 18, 24, and 29 had a mean of 38.6 ± 4.9 (S.D.) primary rays in each labral fan.

***S. tuberosum* cytospecies FG**

Late instars of this multivoltine species were found on 8 May 1985 at site 2 but were outnumbered by AB, five to one. FG was not collected at this location on 26 May 1985. On 23 June 1984, a small population (median, ca. 95% CI = 0 m^{-2}, 0-240 m^{-2}) was found at site 2. Populations of FG also occurred at sites 4, 12, 14, 15, 17, 19, 21-22 (11, 27 June), 27, 28, 30, 31, 33, 36, 38, and 41-43. Watercourses in which FG occurred varied from 1 to 15 m in width, from 10 to 125 μS cm^{-1} in conductivity, from 7.20 to 8.50 in pH, and from 2 to 27 mg l^{-1} in seston. Water temperature rarely exceeded 21°C. Only two larvae were obviously parasitized, both with *C. simulii* (site 22).

Sex-chromosome constitution was St/FG in male larvae (n = 33) and FG/FG in female larvae (n = 59). These characteristics indicated that the FG sibling in Alberta is closer to populations of FG across eastern Canada than to populations in the eastern United States or west of the Rocky Mountains. Approximately 6% of males and females carried B chromosomes, not previously reported for this sibling. One larva (site 43) with B chromosomes was triploid (St/FG/FG); gonadal tissue indicated sex was male.

Larvae of FG were darker than those of AB. In most collections, color afforded a morphological tool for separating FG from AB. Sorting of 33 larvae (15 FG, 18 AB) at site 4 into two color classes (dark, light) and then chromosomally scoring each larva yielded 91% accuracy of identification. Presorting of 71 larvae (6 FG, 65 AB) at site 2 was 100% accurate. Color-based identifications of most other collections of FG, AB, or both, gave excellent results.

Only one collection (site 14) could not be sorted accurately by color. Final-instar larvae (n = 21) from sites 2, 21, and 22 had a mean of 40.8 ± 3.6 primary rays in each labral fan.

***S. tuberosum* AB x FG**

Eight of 33 collections of AB and FG contained both species. However, of 259 AB and FG larvae examined chromosomally, only one unusual sex-chromosome pair was detected. This was an AB/FG combination in a female larva taken at site 28. The stream at this site was 14°C, from three to eight meters wide, and had a pH of 7.78 and a conductivity of 13 μS cm^{-1}. Larvae of the *S. tuberosum* complex (89% FG, 11% AB) were common at this site. Chromosomal homologues exhibited loose pairing.

***S. tuberosum* cytospecies FGH**

Larvae of FGH were collected at sites 13, 20 (15, 27 June), 30, 32-35, and 37 in the Foothill Ecoregion. These streams varied in temperature from 11° to 19°C, in width from 0.3 to 4.0 m, in pH from 7.57 to 8.32, and in conductivity from 8.0 to 40.0 μS cm^{-1}. Seston registered less than 5 mg l^{-1}. Larvae of *Prosimulium* were present in all collections of FGH; larvae of the *S. vernum* group were present in six of nine collections. The FG cytospecies was collected with FGH twice, both times in streams 3 to 4 m in width. The AB cytospecies was not taken with FGH. Voltinism of FGH could not be determined within the time frame of this study.

More than 45% of larvae (n = 75) at site 30 were parasitized by mermithid nematodes. Larvae of *Rhyacophila* (Trichoptera) were observed preying on FGH larvae at this site.

Larvae of FGH were darker than those of FG and AB, but were difficult to distinguish from FG. Final-instar larvae of FGH (n = 17) from sites 20 and 35 had a mean of 41.1 ± 2.8 primary rays in each labral fan. Although there was a progressive increase in the average (and median) number of rays from AB to FG to FGH, the difference between AB and FGH was not significant ($P > 0.05$; Mann-Whitney U Test).

***S. tuberosum* cytospecies FGI**

This species was rarely collected. One male larva was taken with 22 FGH and 7 FG larvae in a stream 3 m wide and 11°C (site 30). Three female larvae were collected with one FGH and two FG larvae in a stream four meters wide and 16°C (site 33). One inversion (limits 44p-53p) was noted in a female larva. This inversion is known from populations of FGI in the Yukon and Norway (Mason 1984).

Larvae were similar to those of AB in head-capsule coloration and to those of FG in abdominal coloration. Small sample size precluded evaluation of pigmentation as a character for separating FGI from FGH, FG, and AB.

***S. venustum* cytospecies CC**

Cytologically classical populations of sibling-pure CC occurred at sites 4 and 22. Middle through ultimate instars were found at site 4 on 2 June; all larvae had pupated by 10 June. Larvae at site 22 were collected on 27 June. Temperature at these sites varied from 12° to 16°C, conductivity from 38 to 108 μS cm^{-1}, stream width from 1.2 to 6.4 meters, and seston from 5 to 24 mg l^{-1}; pH was consistently around 8.30.

Table 3. Larval densities and stream characteristics at site 1, Alberta, from June through July 1984.

	10 June	16 June	23 June	30 June	7 July	14 July	21 July
Larval density[a]							
C. dacotensis	0, 0, 0	0, 30, 50	0, 10, 90	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
S. vittatum IIIL-1	0, 10, 180	430, 1090, 1400	280, 1520, 1960	50, 1540, 1850	0, 2030, 8280	0, 110, 380	0, 390, 990
S. vittatum IS-7	0, 0, 20	0, 0, 50	0, 80, 110	0, 0, 240	0, 0, 1510	0, 0, 0	0, 0, 0
S. decorum	20, 120, 180	10, 730, 2120	0, 40, 160	0, 0, 20	0, 0, 0	0, 0, 0	0, 70, 250
S. venustum CC4	0, 5, 20	0, 0, 10	10, 210, 280	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
S. verecundum ACD	5, 10, 20	0, 10, 20	10, 140, 190	0, 170, 520	0, 110, 2820	0, 60, 190	0, 710, 5540
Temperature (°C)	14.0[b]	11.0–20.0	11.5–21.0	13.0–25.5	11.0–23.0	12.0–23.0	12.0–23.0
Conductivity (μS cm^{-1})	62	34	43	50	47	37	43
pH	NA[c]	7.55	8.09	8.65	8.08	7.90	8.57
Dissolved O_2 (mg l^{-1})	NA	NA	NA	9.0	9.5	7.0	12.3
Minimum width (m)	5.8	3.6	2.4	3.0	2.0	3.6	1.5
Maximum width (m)	8.2	6.4	5.8	5.8	7.6	7.6	7.3
Mean depth (cm)	30	11	10	5	7	11	3
Seston (mg l^{-1})	18	18	31	22	23	47	6
Mean velocity (cm sec^{-1})	41	28	26	20	30	54	15

[a]values are median with ca. 95% CI, except 30 June (minimum, median, maximum).
[b]measured at time of collection; other values are minimum-maximum.
[c]not available.

Table 4. Larval densities and stream characteristics at site 2, Alberta, from June through July 1984.

	10 June	16 June	23 June	30 June	7 July	14 July	21 July	31 July
Larval density[a]								
C. dacotensis	0, 0, 0	0, 0, 10	10, 30, 50	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
S. vittatum IIIL-1	0, 0, 40	0, 20, 510	33, 228, 350	280, 410, 5450	940, 1400, 1860	50, 1205, 2360	0, 160, 230	0, 10, 10
S. vittatum IS-7	0, 10, 40	0, 10, 240	17, 110, 335	0, 20, 250	100, 200, 300	0, 0, 0	0, 0, 0	0, 0, 0
S. decorum	0, 0, 10	0, 30, 2470	20, 140, 180	0, 10, 40	440, 720, 990	80, 910, 1740	520, 5120, 10390	40, 100, 100
S. tuberosum AB	0, 20, 40	0, 70, 290	30, 570, 2000	0, 80, 900	0, 10, 20	0, 0, 0	0, 0, 0	0, 0, 0
S. venustum CC4	0, 0, 10	0, 38, 680	120, 430, 1570	0, 0, 16	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
S. verecundum ACD	10, 10, 70	0, 12, 240	0, 210, 890	0, 80, 450	210, 210, 210	0, 260, 520	0, 20, 20	0, 0, 0
Temperature (°C)	17[b]	12 – 20.5	12 – 21	16 – 23	13 – 21	14 – 23	16 – 23	14 – 24
Conductivity (μS cm^{-1})	65	45	50	70	45	80	80	80
pH	NA[c]	8.35	8.50	7.97	7.80	7.86	7.98	7.99
Dissolved 0_2 (mg 1^{-1})	NA	NA	NA	7.6	10.4	10.1	7.3	6.7
Minimum width (m)	7.6	3.7	2.4	2.0	1.5	1.7	1.5	0
Maximum width (m)	11.3	9.4	7.6	6.1	5.6	5.5	7.3	6.7
Mean depth (cm)	17	14	10	3	3	4	5	8
Seston (mg 1^{-1})	36	17	27	32	22	11	13	18
Mean velocity (cm sec^{-1})	74	61	47	33	11	29	31	46

[a] values from 10 – 30 June are median with ca. 95% CI; values from 7 – 31 July are minimum, median, and maximum.

[b] measured at time of collection; other values are minimum-maximum.

[c] not available.

***S. venustum* cytospecies CC3**

Larvae of this entity were cytologically similar to the CC3 sibling of Rothfels (1981) from northern Québec in that, generally, females were homozygous inverted and males heterozygous for IIIL-5. As in classical CC3, subterminal rearrangements were common in IIIL of males and females. Deviations from classical CC3 included absence of B chromosomes [also lacking from CC3 populations from northern Manitoba (Rothfels pers. comm.)], and rarity of the IIL-1 (grey Band) inversion. At site 38, all six males were heterozygous for IIIL-5, whereas of 13 females, 10 were homozygous inverted, two were homozygous standard, and one was heterozygous. Sex-exceptional females (IIIL-5 = standard) on the order of 2% were observed in populations from northern Québec (Rothfels 1981). Females (from site 38) homozygous standard for IIIL-5 might be members of cytotype CC4 (see next section). Each of six larvae (males and females) from site 38 had a different heterozygous rearrangement in IIIL, with breakpoints between 95c and 100c. Although only one larva (male) large enough for scoring was collected at site 22 (11 June), it was heterozygous for IIL-1 and IIIL-5. These are classical criteria of the CC3 sibling, but are also found in some CC2 populations (Rothfels 1981). Large numbers of early instars with the CC3 larva on 11 June probably represented the CC sibling, since it was the only sibling present on 27 June. Small samples of larvae from sites 19, 22, 23, 27, 28, and 30 were closest to the CC3 sibling in sex chromosomes.

Because larvae from site 38 were most representative of CC3, physical and chemical characteristics are given for this site only: temperature, 14°C; conductivity, 10 $\mu S\ cm^{-1}$; pH, 7.20; dissolved oxygen, 8.9 $mg\ l^{-1}$; seston, 5 $mg\ l^{-1}$; width, 1.5-6.0 m. Several larvae at site 19 were parasitized by mermithid nematodes.

***S. venustum* cytotype CC4**

This taxon is provisionally established to accommodate larvae from sites 1, 2, 3, and 6 that deviated from classical CC in the distal region of IIIL. Three female larvae from site 6 were homozygous standard for IIIL-5. Virtually all females (n = 83) and about 90% of males (n = 78) at sites 1-3 (8 May-30 June) were homozygous standard for IIIL-5. Remaining males were heterozygous for IIIL-5. Less than 8% of males and females combined carried a heterozygous, subterminal inversion in IIIL. IIS was always CC, except for one male (IIS = DC) and one female (IIS = AC). An additional male was standard for IIIL-5 but carried IIL-1 heterozygously. That IIIL-5 apparently functions as a Y chromosome in a small proportion of the males suggests affinities with the CC3 sibling of Rothfels (1981); however, in CC3 the standard IIIL-5 sequence is the Y chromosome. Absence of IIIL-5-inverted females argues against the presence of classical CC3. Reproductive isolation from the CC sibling is suggested by lack of females heterozygous for IIIL-5 despite "quasisympatry," pure populations of CC having been found synchronously only 6.5 km from CC4 populations.

Populations at sites 1-3 were bivoltine, the first generation attaining final instar by the second week of May and the second generation disappearing by July. Population dynamics of larvae and associated physical and chemical parameters at sites 1 and 2 are given in Tables 3 and 4. Only one larva from collections of CC4 was obviously parasitized; a female at site 2 harbored a mermithid nematode.

***S. verecundum* cytospecies ACD**

This multivoltine species was found as early as 12 May (site 8), at which time final instars were already common. Larvae were not found at site 2 on 8 or 26 May 1985, but in 1984 final

instars were collected on 2 June. Larvae were abundant from June through July at sites 1 and 2 (Tables 3, 4). At site 3, 0.1-m^2 samples of 37 and 21 larvae were collected on 24 and 30 June, respectively. Site 4 supported at least three generations: one in early June, one in July (median, ca. 90% CI = 1900 m^{-2}, 820-2470 m^{-2}; as measured 14 July), and another beginning in early August. Additional collections of ACD were made at sites 5 (2 June, 1 July), 6, 11, 29, 31, 36, 38-41, and 44-47. Larvae experienced a wide range of physical and chemical conditions (Tables 2-4). Minimum pH (7.20) and conductivity (10 μS cm^{-1}) were experienced by a large population at site 38. Maximum pH (9.90) was experienced at site 5 (1 July) by a population with a median larval density (with ca. 95% CI) of 50 m^{-2} (0-60 m^{-2}).

At site 1, 0.07% of larvae were infected with a microsporidium, *Thelohania* sp., and 0.2% with *C. simulii*. One larva at site 38 harbored the microsporidium, *Pleistophora multispora*.

Of 341 larvae from central Alberta scored for B chromosomes, approximately 12% were B-positive. The X_3 sequence of chromosome IIL and the CC sequence of IIS predominated in samples from sites 1-4. Morphologically, larvae were extremely variable in color and expression of headspot pattern. This variability prevented morphological separation from larvae of the *S. venustum* complex.

***S. vittatum* cytospecies IIIL-1**

Larvae of IIIL-1 were abundant at sites 1-4. IIIL-1 apparently overwintered as eggs at site 2; a large collection from the first generation of black flies in 1985 (8 May) produced no IIIL-1 larvae. Sixteen days later, a collection at this site yielded many IIIL-1 larvae of all instars. Large numbers of larvae in mid-June probably represented a second generation. I am uncertain as to how many generations occurred from June through July (Tables 3, 4). Larvae of IIIL-1 and all other species at sites 1 and 2 occurred most abundantly on the undersurface of stones. These larvae actively filter-fed *in situ*, as determined by rapid uptake of Day-Glo® pigment released into the current for one-minute periods. Immatures of IIIL-1 experienced a wide range of physical and chemical conditions (Tables 2-4). Additional collections of IIIL-1 were made at sites 5 (1 July), 11, and 26.

Patent microsporidian infections [*Thelohania varians* and *Tuzetia debaisieuxi* (Jírovec)] occurred in 0.4% of larvae at each of sites 1 and 2 and in 3.2% of larvae at site 4. The fungus *Coelomycidium simulii* was detected in 0.6% of larvae at site 1, in 0.3% at site 2, and in 1.6% at site 4. Two male larvae from a small population (median, ca. 95% CI = 0 m^{-2}, 0-140 m^{-2}) at site 5 were infected with mermithid nematodes.

Larvae of IIIL-1 from all sites were highly standard for four of the five major autosomal sequences of Rothfels and Featherston (1981). Of 51 larvae from sites 1 and 2 that were scored for major inversions, the following percentages represented the standard sequence: 100% (IIL-9, IIL-22, IIIL-23), 96% (IIIS-2), 45% (IL-2). The sample contained 5.3% male sex exceptions (homozygous standard for IIIL-1) and 3.0% female sex exceptions (heterozygous for IS-7). Overall, 2.5% of males (n = 324) and 0.9% of females (n = 471) from all sites were sex exceptions. All of these sex exceptions were assigned to the IIIL-1 sibling because they carried the standard sequence for IIL-9, IIL-22, IIIL-23, and IIIS-2.

***S. vittatum* cytospecies IS-7**

Larvae of IS-7 occurred at sites 1 and 2, though in numbers much subordinate to IIIL-1 (Tables 3, 4). At site 1, only 5.6% of larvae of the *S. vittatum* complex were IS-7; at site 2, 9.6% were IS-7. IS-7, like IIIL-1, also appeared to overwinter as eggs at site 2. Larvae were not

found in collections after 7 July at either site. Additional collections of IS-7 were made at sites 5 (16 June, 1 July), 9, 11, 31, 36, 39, 40, 45, and 47. Water temperature rarely exceeded 23°C at any of these sites. Larvae experienced maximum pH (9.90) at site 5. Maximum width of any watercourse with IS-7 was 36 m.

Only 0.5% of larvae at site 2 were infected with pathogens (*Thelohania varians*). Mermithid nematodes were common in male and female larvae of IS-7 from site 5; 82.6% of a small population (median, ca. 95% CI = 10 m^{-2}, 0-190 m^{-2}) was infected on 16 June, and 25.6% of a larger population (median, ca. 95% CI = 100 m^{-2}, 0-1710 m^{-2}) on 1 July.

Cytological parameters of IS-7 larvae from all sites were similar to those recorded by Rothfels and Featherston (1981) for larvae from the Sturgeon River (site 5). In the present study, no sex exceptions were noted at sites 1-2 (n = 103), although two exceptions (one male with nematode, IS-7 homozygous inverted, n = 23; one female, IS-7 heterozygous, n = 27) were recorded from site 5.

***S. vittatum* IIIL-1 x IS-7 ?**

The small proportion of sex exceptions in both cytospecies and the near-fixation of most major autosomal sequences (standard for cytospecies IIIL-1, inverted for cytospecies IS-7) facilitated cytological detection of possible hybrids. The majority of sex exceptions could be assigned readily to either IIIL-1 or IS-7, based on autosomal sequences. Three females from site 1, however, could not be assigned to either cytospecies and, in fact, were intermediates (heterozygous for IS-7, IIL-22, IIIS-2, IIIL-23; standard for IIIL-1, IIL-9). However, chromosomes of these larvae did not show the loose pairing characteristic of other known black fly hybrids (Rothfels and Nambiar 1981).

COMPARISONS

The five species complexes in this study represent at least 12 to 13 cytologically distinct sibling species. Discovery of three of these siblings in Alberta extends the geographic range of each by at least 1500 km. *Simulium tuberosum* FGH previously was known no farther west than Arkansas, *S. tuberosum* FGI only from Alaska, the Yukon, and Norway (Mason 1984), and *S. venustum* CC3 only from northern Québec and northern Manitoba (Rothfels *et al.* 1978; Rothfels pers. comm.). These extensions emphasize the essentially trans-Nearctic distribution of most siblings in this study.

Despite broad geographic overlap of siblings, possible hybrids in Alberta are rare: absent in the *S. aureum* complex and about 0.3% each in the *S. vittatum* and *S. tuberosum* complexes. Over their entire range, the specific distinctness of *S. aureum* A and B remains cytologically intact (Leonhardt 1985). the single AB/FG female of *S. tuberosum* is the first record of an individual having this sex-chromosome combination. Pairings of FGH/FG, absent from this study, are rare throughout eastern North America, only two examples having been recorded (Mason 1984). Possible hybrids between *S. vittatum* IIIL-1 and IS-7, though difficult to detect in most geographic areas because of exceptional sex chromosomes and intermediate frequencies of autosomal polymorphisms, seem rare in Alberta where cytological characteristics produce more distinct categories. Chromosomal variation within the *S. venustum* complex emphasizes the difficulties of unequivocally assigning individual larvae to particular species. Difficulties also occur in classifying populations, for example, at sites 1-3 and 6 where larvae (provisionally designated CC4), otherwise CC, are standard in IIIL or carry IIIL-5 in low frequency

apparently as a Y chromosome. The degree of reproductive isolation within the complex remains unresolved.

Cytological evaluation of species in this study minimizes misinterpretation of variation. *Simulium decorum* in Alberta occurs not only below beaver dams and other impoundments (*e.g.*, site 2), but also in streams lacking such physical attributes (*e.g.*, sites 1 and 3). In eastern North America, *S. decorum* is found almost exclusively below impoundments. Likewise, whereas *Cnephia dacotensis* in Pennsylvania is essentially restricted to the outflow from impoundments (Adler and Kim 1986), in Alberta this association is variable. Cytological criteria indicate that these differences in habitat usage simply reflect biological variation within single, widespread species.

On the other hand, differences in pigmentation among larvae of *S. tuberosum* can be ascribed to separate species. Larvae of FGH are darkest, whereas those of AB are lightest; larvae of FG are intermediate in intensity. The relation of sibling to pigmentation is probably referable, in part, to ecological (temperature?) differences among siblings, although at mixed sites under apparently identical ecological regimes, larvae of AB and FG are still readily assignable to the appropriate sibling.

These three siblings of the *S. tuberosum* complex partition habitat in Alberta. FGH occurs in cool streams less than 4 m in width, whereas AB occurs in productive streams and warm rivers. FG occurs most frequently in streams of intermediate nature and overlaps spatially with both FGH and AB. This is the first example of three sibling species within a complex partitioning habitat along the river continuum. Examples of spatial differentiation between two members of a complex have been documented elsewhere (Adler and Kim 1984, 1986). Ecology of FGI, the fourth Alberta member of the *S. tuberosum* complex, is inadequately known because of the small sample from the province. However, collections of unadulterated FGI with FG and FGH attest to its specific distinctness, and suggest that its ecological requirements might be similar. In the eastern United States, where as many as six or seven siblings of *S. tuberosum* occur and where FG exhibits Y-chromosome polymorphism (Mason 1984), habitat relationships among these three siblings (AB, FG, FGH) are less clear than in Alberta (Adler unpublished).

Simulium vittatum IIIL-1 and IS-7 in Alberta are similar ecologically to populations in eastern North America (*cf.* Adler and Kim 1984). For example, in warm, organically enriched streams, IIIL-1 achieves large populations, whereas IS-7 is present in much smaller numbers and disappears as the season progresses. IS-7, however, is found in wide rivers more commonly in Alberta than in the eastern United States. The additional inversions associated with the Y chromosome of this sibling in Alberta (Rothfels and Featherston 1981) might confer some environmental flexibility over eastern populations. Also, streams and rivers in much of Alberta might not maintain extremes (*e.g.*, of temperature) long enough to stress larvae of IS-7.

Simulium verecundum ACD is ecologically distinct from the *S. venustum* complex in Alberta. After June, only ACD is present in the Parkland and Mixedwood Ecoregions. ACD generally completes three or more generations annually, whereas the *S. venustum* lineage completes one or two in these regions. ACD also experiences warmer temperatures (>20°C) than siblings in the *S. venustum* complex. These findings are similar to those in eastern North America. *Simulium verecundum* ACD is multivoltine in New York and Ontario, first appearing as larvae at least as early as May (Rothfels *et al.* 1978; Gordon and Cupp 1980). *S. venustum* CC in the east is typically univoltine although in certain habitats it is multivoltine (Adler and Kim 1986). Watercourses in which ACD occurs in Alberta are higher in pH than

those sampled in New York. This difference is probably an artifact of limited sampling and, in reality, suggests a wide tolerance range for this species. Presently, it is unclear why *S. verecundum* ACD, which in sympatry consistently occurs in warmer flows than the *S. venustum* complex, should be restricted north of the 45th parallel while *S. venustum* CC occurs southward into Louisiana.

Within the *S. venustum* complex, ecological segregation of CC, CC3, and CC4 is unresolved, although there is some evidence that larval populations of CC and CC3 in the same stream (site 22) are out of phase. Siblings responsible for human biting in Alberta remain unknown, but are present in the Foothill, Mixedwood, and Parkland Ecoregions. A third member of the *S. venustum* lineage, *S. truncatum* Lundström, probably occurs in the province during the early part of the season. Ecological differences among siblings in the *S. aureum* complex also remain largely enigmatic, although because sibling-pure populations have been found (Adler and Kim 1986; Leonhardt 1985), differences must certainly exist.

Coupling cytological evaluation with ecological and morphological studies of black flies, as in this investigation, provides an effective means of delimiting variation within species. The value of this approach is explicit in studies of black flies where variation often occurs within a morphospecies at a single site and over a wide geographic area.

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EVIDENCE FOR GENETIC CONTROL OF SEX RATIO DISTORTION IN TWO COLONIES OF *GLOSSINA MORSITANS SUBMORSITANS* NEWSTEAD (DIPTERA: GLOSSINIDAE)

R. H. Gooding
Department of Entomology
University of Alberta
Edmonton, Alberta T6G 2E3
Canada

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ABSTRACT

Males of Glossina morsitans submorsitans *Newstead sired families which had only females or which had both males and females (with males usually comprising 20 to 70% of the offspring). The phenomenon occurs in two colonies, one originating near Komoé, Burkina Fasu, and the other from Yankari Game Preserve, Nigeria. A single generation of selection for or against sex ratio distortion, using flies from both colonies, resulted in shifting the sex ratio in the expected direction. The data indicate that sex ratio distortion in* G. m. submorsitans *is controlled by an X chromosome-linked factor. A breeding program designed to increase the proportion of males in a colony is proposed.*

RÉSUMÉ

Des mâles de Glossina morsitans submorsitans *Newstead ont produit des progénitures comprenant seulement des femelles, ou bien des progénitures mixtes mais dont les mâles représentaient généralement de 20 à 70% du total. Ce phénomène a lieu dans deux colonies, l'une provenant des environs de Komoé au Burkina Fasu, et l'autre provenant de la réserve faunique de Yankari au Nigéria. En effectuant avec des mouches des deux colonies une sélection pour ou contre ce déséquilibre du rapport des sexes, une seule génération suffit pour ramener le rapport des sexes dans la direction prévue. Les données indiquent que le déséquilibre du rapport des sexes chez* G. m. submorsitans *est sous le contrôle d'un facteur lié au chromosome X. L'auteur propose un programme d'élevage visant à accroître la proportion des mâles d'une colonie.*

INTRODUCTION

In most animal populations there are equal numbers of males and females. This is believed to result from the selective advantage that would accrue to an individual producing offspring of the sex which is in short supply since such an individual would have a greater chance of having "grandchildren" than would an individual producing offspring of the sex which is in excess. (For a recent discussion of this see J. Maynard Smith, 1978.)

Among the exceptions to occurrence of equal numbers of males and females are colonies of *Glossina morsitans submorsitans* Newstead, which originated from Burkina Fasu (Gooding, 1984; 1985) and from Nigeria (Gooding, 1984; 1985; Rawlings and Maudlin, 1984). In these colonies an excess of females occurs because some males sire daughters only, while others sire both sons and daughters (Rawlings and Maudlin, 1984). Rawlings and Maudlin (1984) presented no experimental evidence concerning the genetic mechanism(s) by which sex ratio distortion occurs in *G. m. submorsitans*, but they alluded to "experiments in progress" which indicated that "temperature stress and decreased frequency of feeding can [change] the sex

ratio towards normality".

As well as being of theoretical interest, knowledge of the mechanism(s) of sex ratio distortion in *G. m. submorsitans* may be of practical value. Where sterile males are released as part of an integrated control program, sex ratio distortion resulting in an excess of females will increase the size of the colony necessary for production of a given number of males and thus increase the cost of each sterile male released. Environmental manipulations or breeding programs which increase the proportion of males in a colony of *G. m. submorsitans* could contribute to the cost effectiveness of an S.I.T. program for this species.

Because of the dearth of published information on the mechanism(s) of sex ratio distortion in *G. m. submorsitans*, the present study was undertaken to determine whether the tendency of males to sire families with a distorted sex ratio is inherited. Here I compare the magnitude of sex ratio distortion in two colonies of *G. m. submorsitans*, I present evidence that the tendency for males to sire families with a distorted sex ratio is inherited as an X chromosome linked trait, and I propose a breeding program designed to create a line of *G. m. submorsitans*, with an increased proportion of males.

MATERIALS AND METHODS

Two colonies of *Glossina morsitans submorsitans* Newstead were maintained at approximately 24°C by feeding on rabbits five or six days per week. In this paper the colony which originated from Komoé, Burkina Fasu, is designated Gms(BF) and the one originating from Yankari Game Preserve, Nigeria is designated Gms(N). The histories of these colonies were described previously (Gooding 1982; 1985).

Sex ratio among offspring of each male was determined as follows. Each male was placed in a cage with five or six virgin females from the same colony and the flies were maintained as indicated above. (Females in each cage emerged within a three day period and thus could not be full-sibs, but any female in a cage could have been a full-sib of the male placed in that cage and she could be the half-sib of any of the other females in the cage.) Puparia were collected at intervals until at least 11 were produced by each cage of females. The sex of each emerging adult was recorded. Progeny of a single male are referred to as a "family". Data are presented for those families in which 11 or more adults were obtained.

F_1 females from those families which had no males were mated individually with colony males, from the appropriate colony, and placed in cages designated "Gms(BF) distorter" or "Gms(N) distorter". (To set up "Gms(BF) distorter" 55 females, from the first four families listed in column 1 of Table 1, were used. For "Gms(N) distorter" 77 females, from the first five families listed in column 1 of Table 2, were used. The females in each family were at least half-sibs and since the father of each female had been with five or six females, between 16.7 and 20% of the females in each family were full-sibs.) Male offspring thus obtained (designated Gms(BF)-D-F_2 and Gms(N)-D-F_2) were mated with several colony females and the sex ratio among their offspring was determined as indicated above.

F_1 females from those families which had both males and females were mated individually with males from these families (designated Gms(BF)-N-F_1, or Gms(N)-N-F_1) and maintained as above. (To set up Gms(BF)-N-F_1 88 females from the last 11 families listed in column 1 of Table 1 were used, whilst for Gms(N)-N-F_1 143 females from the last 16 families in column 1 of Table 2 were used. Sib-ships are the same as stated in the above paragraph.) The sex ratio in families sired by their sons (i.e. Gms(BF)-N-F_2 and Gms(N)-N-F_2 males) was determined as